THE EFFECT OF ACIDS AND ALKALIS ON THE GROWTH OF THE PROTO-PLASM IN POLLEN TUBES

BY FRANCIS E. LLOYD*

McGill University

In 1915 it was shown† that the protoplasm of pollen grains acts in the presence of acids, alkalis, and salts in general accordance with the behavior of the biocolloid gelatin. The evidence then secured appears to indicate that the amount of swelling is greater in acids than in alkalis, and less in salts than in pure water, from which, in the light of MacDougal's experiments, the preponderating protein component of the complex may be inferred.‡ It soon became evident, however, that the amount of swelling for various concentrations of solution of the reagents used was not constant, and it was found necessary to determine this relation for an assumed analog, gelatin, and then to find the material and method by which the comparative behavior of the protoplast in the living condition could be studied. The results of the measurements of the swelling rates of gelatin in a number of acids and alkalis, and these in combination with certain salts have been reported upon in general form.§ These are in brief as follows:

1. The swelling rates differ in both acids and alkalis for different concentrations. The maximum rates are found at certain concentrations above *ca.* $N/640$, which are higher for organic acids than for inorganic acids. At higher concentrations "repression" || occurs. The higher rates are at first at higher concentrations, but as time elapses the rates at successively lower concentrations

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† Lloyd, F. E. Carnegie Inst. Wash. Ann. Rep. for 1915.

‡ MacDougal, D. T. Science II. **44**: 502. 6 O 1916.

——— & Spoehr, H. A. Science II. **45**: 484. 18 My 1917.

§ Lloyd, F. E. Trans. Roy. Soc. Canada, 1917 (In press).

|| Procter, H. R., & Wilson, J. A. Jour. Chem. Soc. London **109–110**: 307–319. 1916.

overtake them. At certain concentrations below *ca.* *N*/640, *the rates are lower than those for pure water*, but much more markedly so for acids than for alkalis.

2. The swelling rates and total swelling in acids is greater than in alkalis.

3. The swelling rates and total swelling is greater in some organic acids (citric, malic) than in inorganic acids.

Acetic and tartaric acids appear to be excepted. Malic acid has a far-reaching effect on gelatin in causing it to fragment at *N*/160 and above after one to two hours. This fact, together with the lower swelling rates at the higher concentrations, suggests that at these there is a coagulation effect which sets in to repress swelling.

It thus appears that in trying to establish any analogy between gelatin (or other emulsoid) and protoplasm, *the concentrations of the reagents to which they are subjected must be considered.* For example, during the increase or decrease of acidity which may take place in the living tissues, the swelling effects may be alternately repressed and increased, aside from alterations in the relative composition of the body fluids due to change in salt, protein, or other content, such as MacDougal has indicated.*

The determination of growth rates and accompanying phenomena in pollen tubes confirms the expectation that their protoplasm behaves toward the above reagents in many important respects as does gelatin rather than agar.† The method employed consists in sowing pollen of *Phaseolus odoratus* in hanging drops of the various reagents at different concentrations, associated with cane sugar in constant concentration, it having been found‡ that the rate of growth of pollen tubes is inversely as the concentration of cane sugar, the maximum accomplished growth occurring in *ca.* 20 per cent solution. It has been shown that this is explainable on the assumption that imbibition by the protoplasm rather than osmotic pressure is the dominant growth factor. In weaker solutions of cane sugar the pollen tubes burst, the lower the concen-

* MacDougal, D. T. Science II. **46**: 269. 14 S 1917.

† The contrary has been found to hold for complex tissues such as cactus stems by MacDougal and Spoehr. Proc. Amer. Phil. Soc. **56**: 289. 1917 (and other already cited papers).

‡ Lloyd, F. E. Carnegie Inst. Wash. Ann. Rep. for 1916.

tration the more quickly, and for this reason little total growth can be attained, although the initial rates are higher than that at higher concentrations. The cultures were run in a double series, and the concentrations of acid and alkali were varied between *N*/25,600 and *N*/100.

During the course of experimentation it was found that positive results in terms of growth could be obtained with alkali when associated with 20 per cent cane sugar, but that acids so associated caused the pollen grains or tubes to burst. This again indicated the greater swelling effect of acid over alkali. It was then found that by increasing the concentration of cane sugar to 40 per cent, the effect of acids was held in check and that growth proceeded, bursting taking place only at certain concentrations of acid, but in the lower of these only after a certain amount of growth had been attained. With this difference understood, it was shown that the behavior of the growing protoplasm was otherwise and in general the same toward both acids and alkalis. Summarily stated it is as follows:

At certain concentrations of the reagent, growth proceeds more rapidly than in the control, namely, the pure cane-sugar solution. The maximum growth occurs for acetic acid at *N*/3200, for malic acid at *N*/12,800, and for citric acid at *N*/12,800, or perhaps less. Hydrochloric, formic, and oxalic acids did not afford positive results in terms of growth, and indeed the evidence for citric acid was not unequivocal. This was not because they did not produce increased imbibition in the protoplasm, but probably because of pathological results which militated against the attainment of growth. It is important to note that for those acids which gave the data sought, there was less growth for concentrations above and below the ones just indicated, and in this we may see a correspondence with gelatin in its maximum swelling response to certain concentrations already mentioned. The correspondence is heightened in the growth rates which in low concentrations of the reagents are lower than in the control. It was previously shown that essentially the same behavior occurs in alkali, sodium hydrate having been used.

That a higher concentration of cane sugar must be used with acids may be due to the already acid condition of the protoplast.

Growth took place in alkali also in a greater range of concentrations, namely, from *N*/400 to *N*/25,600, and it was determined that the Na-ions penetrated the protoplast. In *N*/400 the growth was less than in the control, this, it is possible, being related to an increase in salts formed. At all other concentrations the amount of growth was greater than in the control, increasing from the lowest concentration used to *N*/3200 and falling for those still higher.

The failure to obtain positive results with certain acids in terms of growth, as above stated, need not, indeed should not, be interpreted except as indicating that other effects antagonistic to normal behavior intervene.

We may note especially the bursting of the protoplast beyond the confines of the cell wall. The weakest point in the pollen-tube wall is at the apex, and it is here that bursting takes place if it has not already occurred before growth begins. Bursting is due to the imbibition of the protoplast beyond the strength of the wall to confine it, and not, as might be expected, to any change in the wall itself, such as hydrolysis, since the bursting takes place more rapidly at concentrations of the reagent which would cause less hydrolysis.

In acids the bursting takes place within a certain range of concentrations, namely, those above that at which maximum growth takes place and below those at which syneresis of the protoplasm is caused. Syneresis is quite evident in all the acids studied at concentrations at or above *N*/3200, and it is of more than passing importance that syneresis occurs in formic, oxalic, and hydrochloric acids at lower concentrations (*N*/3200 to *N*/1600) than in citric, malic, and acetic acids, and was not observed at all in alkali. It is evident in the course of a short time in the highest concentrations (*N*/800 to *N*/400) but ensues more slowly in the lower effective concentrations. It should be stated that in all these the protoplast swells fully when first subjected to them, completely distending the pollen-grain walls. It then slowly shrinks.

At the close of shrinkage it can be shown that the protoplasm is coagulated, for on pressure it breaks out as a cheesy mass. In this connection it is important to note that at the higher concentrations at which bursting occurs, the protoplasm oozes out of the broken pollen tube in strings, in such a manner as to show that it has a much higher viscosity than has the protoplasm exposed to the same reagent at lower concentrations but which nevertheless cause bursting.

It will thus be seen that the maximum swelling of the protoplasm is at the concentration of the reagent which causes the most rapid bursting, and this is higher than the concentration which causes swelling which can be utilized in growth. The former is chiefly a physical result, the latter physiological. A glance more particularly at the behavior of pollen protoplasts toward malic acid may be taken, this serving as a typical example.

At concentration	There occurs:
N/400	Coagulation and complete syneresis in the course of an hour of all the pollen grains (100 per cent.).
N/800	The same but more slowly and less completely (95 per cent.).
N/1600	50 per cent. of the pollen grain shrinks in the course of 3 hours, the remainder having burst; the protoplasm highly viscous, bursting in strings after some growth (0.3 unit).
N/3200	Bursting of 90 per cent. after 1–1.5 units growth attained; viscosity of protoplasm lower than above but still showing coagulation ("clots").
N/6400	3.5 units growth in 5 hours. Some bursting but no coagulation. 22 hours later: alive, no further growth.
N/12,800	8 units growth in 5 hours, no bursting; 22 hours: 16 units growth, alive.
N/25,600	4 units growth in 5 hours; 22 hours: 5 units growth, alive.
Control:	4.5 units growth in 5 hours; 22 hours: 5 units growth, alive.

In formic acid, bursting unaccompanied by coagulation occurred in *N*/25,600 (with 20 per cent cane sugar) after one unit of growth was attained. Partial coagulation occurred in *N*/6400, more in *N*/3200 and complete in *N*/1600.

The above results indicate that the protoplasm of pollen grains is affected by acids and alkalis in the same fashion as gelatin, and that the increased swelling caused by such reagents can actually be used in growth. The extreme sensitiveness of this protoplasm to low concentrations of acids and alkalis, as evidenced in coagulation and syneresis in the higher, and in the swelling and growth in the lower, is to be noted. It has become patent that the mechanism of growth in more complex plants includes emulsoids which exhibit swellings at much higher concentrations of acids and alkalis* and a final analysis of their relations to growth must, as it will, include the behaviors of these emulsoids. Similarly in the animal body, so far as studied in these relations, it is impossible to analyze the phenomena, and to separate that which occurs in substances extraneous to the protoplasm (*e. g.*, sarcolemma), and that which occurs in the protoplasm itself.

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* Long, E. R. Bot. Gaz. **59**: 491. 1915; MacDougal & Spoehr, as above cited; Fischer, M. Oedema, 1910.